Developmental Biology **205,** 111–128 (1999)
Article ID dbio.1998.9109, available online at http://www.idealibrary.com on IDEAL®

Ovulation in *Caenorhabditis elegans*

James McCarter, Bart Bartlett, Thanh Dang, and Tim Schedl[1]
Department of Genetics, Washington University School of Medicine, St. Louis, Missouri 63110

Prior to fertilization, oocytes undergo meiotic maturation (cell cycle progression) and ovulation (expulsion from the ovary). To begin the study of these processes in *Caenorhabditis elegans*, we have defined a time line of germline and somatic events by video microscopy. As the oocyte matures, its nuclear envelope breaks down and its cell cortex rearranges. Immediately thereafter, the oocyte is ovulated by increasing contraction of the myoepithelial gonadal sheath and relaxation of the distal spermatheca. By systematically altering the germ cell contents of the hermaphrodite using mutant strains, we have uncovered evidence of four cell–cell interactions that regulate maturation and ovulation. (1) Both spermatids and spermatozoa induce oocyte maturation. In animals with a feminized germline, maturation is inhibited and oocytes arrest in diakinesis. The introduction of sperm by mating restores maturation. (2) Sperm also directly promote sheath contraction. In animals with a feminized or tumorous germline, contractions are infrequent, whereas in animals with a masculinized germline or with sperm introduced by mating, contractions are frequent. (3 and 4) The maturing oocyte both induces spermathecal dilation and modulates sheath contractions at ovulation; dilation of the distal spermatheca and sharp increases in sheath contraction rates are only observed in the presence of a maturing oocyte.

Key Words: **oocyte maturation; meiosis; ovulation; cell–cell interaction; sperm; myoepithelial contraction and relaxation; *Caenorhabditis elegans*.**

INTRODUCTION

Oocyte maturation is a cell cycle event which releases the oocyte from meiotic prophase (similar to a G2 to M phase transition) and allows progression through the meiotic divisions and fertilization (Masui and Clarke, 1979; Wickramasinghe and Albertini, 1993; Downs, 1995). Nuclear envelope breakdown (NEBD or germinal vesicle breakdown) is its most characteristic feature. Maturation is preceded by oocyte development (oogenesis), and is rapidly followed by ovulation, releasing the oocyte from the ovary.

Stimuli which can induce oocyte maturation are known in some animals. Maturation is induced by application of 1-methyladenine in starfish (Kanatani *et al.*, 1969) and serotonin in clams (Krantic *et al.*, 1991). In vertebrates, increased release of gonadotropins by the pituitary signals the somatic cells of the follicle for maturation. In some vertebrates, such as amphibians, progesterone is then produced by follicle cells and apparently signals the oocyte to mature (Masui, 1967). In mammals, the mechanism by which the follicular granulosa cells cause oocyte maturation is unresolved, but may involve overcoming inhibition by cAMP (Eppig, 1993). In the genetic model systems *Caenorhabditis elegans* and *Drosophila* the molecular signals for oocyte maturation are unknown.

The study of *Xenopus* oocyte maturation (Masui and Markert, 1971) led to the isolation of maturation promoting factor (MPF), a cyclin B/Cdc2 kinase complex which acts within the oocyte as the crucial regulator of maturation (Lohka *et al.*, 1988; Maller *et al.*, 1989). Activated MPF phosphorylates targets responsible for NEBD and other events which mark the transition from meiotic prophase to metaphase. Cyclins and cyclin-dependent kinases are evolutionarily conserved and play crucial roles in cell cycle regulation of both mitosis and meiosis in organisms from yeast to humans (Nurse, 1990). Studies in *Xenopus* and mouse also indicate a role for the kinase Mos and the MAP kinase cascade in oocyte maturation and/or the transition from meiosis I to meiosis II (Sagata *et al.*, 1989; Kosako *et al.*, 1994; Verlhac *et al.*, 1994).

[1] To whom correspondence should be addressed at Department of Genetics, Washington University School of Medicine, Box 8232, 4566 Scott Ave., St. Louis, MO 63110. Fax: (314) 362-7855. E-mail: ts@genetics.wustl.edu.

0012-1606/99 $30.00

TABLE 1
Strains Used in This Study

Linkage group[a]	Strain	Reference[b]
LG I	N2 (wild-type reference strain, *Bristol*)	—
	BS390 *fog-1(q180)unc-13(e51)/hT2*	Barton and Kimble, 1990
	CB51 *unc-13(e51)* and CB1091 *unc-13(e1091)*	—
	BS1 *spe-4(q347)/unc-109(n499sd)*	L'Hernault *et al.*, 1988
	BS146 *gld-1(q266)/hT2*	Francis *et al.*, 1995
	BS406 *unc-13(e51)gld-1(q485)fog-3(q443)/hT2*	Francis *et al.*, 1995
	BS273 *unc-13(e51)gld-1(oz10)/unc-55(e402)*	Francis *et al.*, 1995
	BA76 *fer-1(hc13); him-5(e1490)*	Ward and Miwa, 1978
	SL154 *fer-1(b232ts); him-5(e1490)*	S. L'Hernault, personal communication
	SL170 *fer-1(hc47)dpy-5(e61)/hT2; him-5(e1490)*	S. L'Hernault, personal communication
	BS3075 *unc-13(e51)fog-3(q443)hT2*	Ellis and Kimble, 1995
	BS196 *fog-3(q443)/lin-10(e1439)unc-29(e193)*	Ellis and Kimble, 1995
	BS3158 *glp-4(bn2); him-5(e1490)*	Beanan and Strome, 1992
LG II	CB120 *unc-4(e120)*	—
	BA3 *fer-3(hc3)*	Argon and Ward, 1980
LG IV	CB138 *unc-24(e138)*	—
	BS3046 *unc-24(e138)fem-3(e1996)/DnT1*	Hodgkin, 1986
	BS3147 *fem-3(e1996)/DnT1*	Hodgkin, 1986
	JK816*fem-3 (q20gf)*	Barton *et al.*, 1987
LG V	JK574*fog-2(q71)*	Schedl and Kimble, 1988

[a] Strains are organized by the linkage group of the gene of interest to this study.
[b] References for the genes of interest to this study are provided. References for all other genes are provided in Hodgkin (1997).

Ovulation is the physical process whereby the oocyte is released from the ovary and becomes available to sperm for fertilization. Ovulation is temporally coupled to maturation in most metazoans, although its features are less conserved. For instance, vertebrate ovulation requires the rupture of the ovarian wall by the follicle in a proteolytic cascade comparable to the inflammatory response (Espey and Lipner, 1994), whereas in *C. elegans* and *Drosophila* myoepithelial sheath cells in the ovary contract at ovulation to move the oocyte out through a passage which directly connects to the spermatheca and uterus (King, 1970; Hirsh *et al.*, 1976). In *C. elegans* the distal spermathecal cells form a stricture at the proximal end of the ovary (or gonad arm) and only dilate to let the oocyte pass at ovulation.

C. elegans is an excellent model for addressing the regulation of oocyte maturation and ovulation. The hermaphrodite has a female soma with a germline that makes sperm first and then produces oocytes in both gonad arms. Full-grown oocytes mature and are ovulated and fertilized in a single file and assembly-line-like fashion. In addition to its utility as a genetic system (Hodgkin *et al.*, 1988) and defined cellular lineages (Kimble and Hirsh, 1979), the worm is transparent and can be anesthetized (Kirby *et al.*, 1990) to allow video recordings of late oogenesis, oocyte maturation, and ovulation. Defective ovulation has already been examined by several groups; disruption of the gonadal sheath's contractile function by laser ablation (McCarter *et al.*, 1997) or mutation (Myers *et al.*, 1996; Iwasaki *et al.*, 1996; Rose *et al.*, 1997) leads to failure of ovulation and sterility with mature oocytes trapped in the gonad arm.

Using time-lapse microscopy with wild-type worms, we have defined a time line of landmark morphological events during oocyte growth, maturation, and ovulation in *C. elegans*. By manipulating the presence or absence of oocytes and sperm in the worm using mutant strains and matings, we have obtained evidence of four cell–cell interactions that regulate maturation and ovulation in this species.

MATERIALS AND METHODS

Nematode Strains, General Methods, and Terminology

Methods for *C. elegans* culture and manipulation were as described (Sulston and Hodgkin, 1988). Strains were grown at 15 or 20°C. Observations and video recordings were made at room temperature (20–23°C) unless otherwise noted. Strains used are listed in Table 1.

The following definitions used here are based on the events that can be visualized by Nomarski microscopy and the reproductive biology of *C. elegans* (also see Results and Discussion). Meiotic maturation: the transition from diakinesis of prophase I to metaphase of meiosis I. Events include nuclear envelope breakdown (NEBD), which begins in the gonad arm prior to ovulation and finishes in the spermatheca, and oocyte cortical rearrangement, which occurs in the gonad arm prior to ovulation. Oocyte: female germ cell up to the time of fertilization. Egg: zygote after the point of fertilization, which is normally followed by egg shell formation.

This definition of egg/embryo includes the possibility that *C. elegans* maternal effect embryonic lethal mutations (Kemphues and Strome, 1997) may exist where eggs are produced (fertilization and egg shell formation) and yet events of meiotic maturation (i.e., NEBD) are defective.

Nomarski and Fluorescence Microscopy

Observations of living animals by Nomarski (DIC) microscopy were as described (Sulston and Hodgkin, 1988) using a Bmax-60F (Olympus, Inc.) microscope. For fluorescence microscopy, nematode gonads were dissected, fixed, and stained as described (Francis *et al.*, 1995). Images were collected using an Optronics DEI-470 cooled CCD camera, transferred to a Power Macintosh 7100 (Apple, Inc.) running NIH Image 1.58 (Wayne Rasband, NIH), assembled with Photoshop 3.0 (Adobe, Inc.), and printed on a Phaser 440 dye-sublimation printer (Tektronix, Inc.).

Nomarski Time-Lapse Microscopy

For time-lapse observations, worms were anesthetized for 30–45 min in a solution of M9 with 0.1% tricaine and 0.01% tetramisole (Sigma, Inc.) before viewing (Kirby *et al.*, 1990; McCarter *et al.*, 1997). Tricaine/tetramisole blocks body wall movement, pharyngeal pumping, and egg laying. Events of late oogenesis, oocyte maturation, ovulation, and fertilization all continue undisturbed for the first 4–5 oocytes in the arm, while new oocytes are not formed at the loop of the gonad arm (possibly because nutrient availability from the intestine diminishes). Worms can be recovered from an anesthetic exposure of up to 4 h. Animals were mounted on an Axioskop (Zeiss, Inc.) microscope and viewed with low light using the 40× or 63× lens. To prevent heating, an infrared filter was added to the light path. The microscope was connected to a XC-75 CCD video camera (Sony, Inc.) or a DEI-470 cooled CCD video camera (Optronics, Inc.). Time-lapse recordings were made at 1/12 real time.

Quantitation of Maturation Rates, Oocyte Volume, Oocyte Nuclear Volume, and Sheath Activity

Maturation is the rate-limiting step in the production of fertilized eggs in adult hermaphrodites (see Results). The rate at which oocytes undergo maturation was calculated by measuring total embryo production in adult hermaphrodite populations and by direct observation of individual animals with video microscopy. For both wild-type and mutant strains the two methods were in excellent agreement. To measure total embryo production in a population, animals were synchronized as described (McCarter *et al.*, 1997). Animals were mounted in M9 for Nomarski microscopy and the total number of embryos in the uterus of each animal was determined. Animals were transferred to fresh plates with *Escherichia coli* for several hours. Upon completion of the time interval, embryos in the uterus and laid on the plate were counted. The oocyte maturation/ovulation rate per gonad arm per hour = (number of embryos at end of interval − number of embryos at beginning of interval)/(2 × population size). The average population size used per trial was 19 and average time interval was 3.5 h.

Oocyte and oocyte nuclear volume were calculated by direct measurement of oocyte width and length, and nuclear diameter on the monitor during video production. Sizes were calibrated to convert cm on the monitor to true micrometer values. We assumed the oocyte nucleus to approximate a true sphere so that volume = $4/3 \times \pi \times (\text{diameter}/2)^3$. We assumed the oocyte cell to approximate a true cylinder so that volume = $\pi \times \text{length} \times (\text{width}/2)^2$. Sheath contractile activity was quantitated by replaying recorded videos and visually counting the number of contractions occurring in the myoepithelium over 1-min intervals. Contractions were counted twice and averaged.

RESULTS

C. elegans Oocytes Are Produced in an Assembly-Line-like Process That Reaches a Steady-State Rate in the Adult Hermaphrodite

The *C. elegans* hermaphrodite reproductive system is made up of two gonad arms, each connected by a spermatheca to the common uterus; gametogenesis occurs in the arms and fertilization in the spermatheca. Oocytes are produced and leave the gonad in a single-file assembly-line-like process so that following maturation, ovulation, and fertilization of the most proximal (first) oocyte, the second oocyte takes the most proximal position, and the third takes the position of the second, etc. (Fig. 1).

The production of fertilized eggs includes the processes of oocyte growth and development, maturation, ovulation, and fertilization, and theoretically any one of these steps could be rate limiting. Examination of oocytes by time-lapse video Nomarski microscopy reveals that in adult hermaphrodites, oocyte maturation is the rate-limiting step with ovulation and fertilization immediately following each maturation. Oocytes mature at a rate of 2.7 ± 1.3 per gonad arm per hour, or one every ~23 min (range 11 to 42 min, $n = 19$ oocytes). This rate agrees closely with the rate of embryo production determined for unanesthetized adult animals on plates. In young adult hermaphrodites producing their first several oocytes, oocyte growth and development prior to maturation is rate limiting with only 1.3 ± 0.3 oocytes produced per gonad arm per hour, or one every ~47 min (range 34 to 83 min, $n = 17$ oocytes). Young adults have only ~5 or fewer oocytes in the proximal arm, whereas mid-stage adults can have a backlog of ~10 oocytes filling the proximal arm and loop. Further, oocytes in young adults are still increasing in size as they near the most proximal position, whereas oocytes in adults reach their full size earlier in their progression through the proximal arm.

Oocyte Development, Meiotic Maturation, and Ovulation Are Defined by Reproducible Landmark Events

The events of late oogenesis, meiotic maturation, and ovulation in the adult hermaphrodite are depicted for one oocyte along a time line in Fig. 2 and presented as individual micrographs in Fig. 3. Completion of ovulation with the closure of the distal spermatheca is defined as the reference zero time point and corresponds closely to the time of fertilization. (Prior events receive negative time values.) Table 2 presents the average time for each event (± standard deviation) and additional features of the events. A

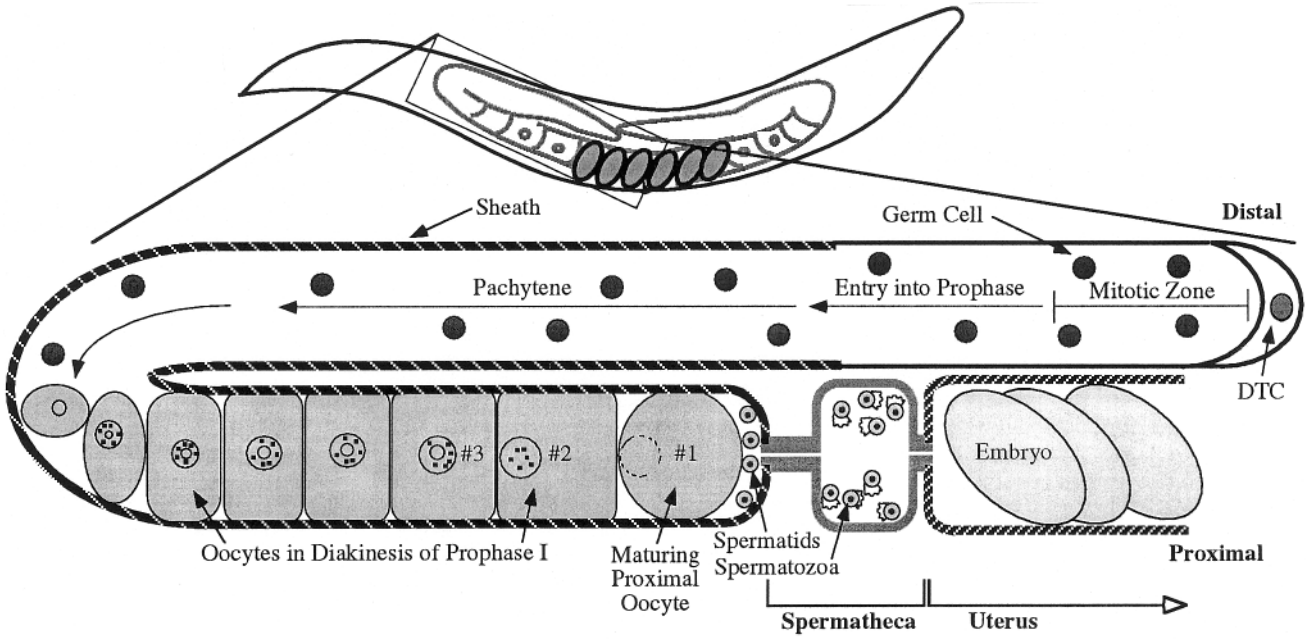


FIG. 1. Schematic of a *C. elegans* adult hermaphrodite gonad arm. Each of the two gonad arms is a U-shaped tube that generates male and female gametes. Germ cells proliferate distally and then enter and progress through meiotic prophase as they move proximally. The first ~40 germ cells to initiate meiotic development undergo spermatogenesis in the proximal region of each gonad arm and complete the divisions of meiosis I and II to form ~160 spermatids. Spermatids mature to spermatozoa and become capable of fertilization only after entering the spermatheca. All subsequent germ cells that initiate meiotic development acquire the female fate. Female germ cell nuclei (presumptive oocytes) proceed through the pachytene stage of meiotic prophase in the distal arm and progress from diplotene to diakinesis from the loop through the proximal arm. Moving through the loop, oocytes become more fully enclosed by membrane and grow in size. Distal germ cells are syncytial, while membrane enclosed diakinesis stage oocytes can remain connected to the syncytium by a narrow rachis (not shown) (White, 1988). Maturation occurs in the most proximal oocyte in the gonad arm. Ovulation then transports the mature oocyte into the lumen of the spermatheca where fertilization occurs. The fertilized egg moves from the spermathecal lumen to the uterine lumen through the spermathecal–uterine valve. Fertilized eggs complete the divisions of meiosis I and II in the uterus. Following maturation and ovulation of the most proximal oocyte (No. 1), oocyte 2 moves to the most proximal position in the gonad arm and will undergo maturation and ovulation over the course of the next ~23 min. The tubular gonad arm is covered by 10 somatic sheath cells, the 6 most proximal of which are myoepithelial and capable of contraction. The lumen of the gonad arm connects proximally to the lumen created by the 24 cells of the somatic spermatheca. The contractile spermatheca can be considered to be myoepithelial as actin filaments are arrayed in a circumferential lattice (Strome, 1986b), and recently an unconventional myosin that is expressed in it has been identified (Baker and Titus, 1997; J. Baker and M. Titus, personal communication). Distal spermathecal cells form a constriction, preventing oocytes from exiting the gonad arm until ovulation when they dilate. Germline development is reviewed in Kimble and Ward (1988) and Schedl (1997). Somatic structures have been described previously (Hirsh *et al.*, 1976; Kimble and Hirsh, 1979; Strome, 1986b; Creutz *et al.*, 1996; McCarter *et al.*, 1997; Rose *et al.*, 1997).

brief description of these events was first reported in Ward and Carrel (1979).

Late oogenesis. Late oogenesis occurs primarily during diakinesis of meiotic prophase I in the proximal gonad arm. At about −77 min, while the developing oocyte is between the second and fourth position in the proximal arm, the nucleolus disappears (Fig. 3a), coinciding with decreased rRNA transcription (Starck *et al.*, 1983). Disassembly of an organized nucleolus is a common feature of both mitotic and meiotic divisions (Busch and Smetana, 1970). As the oocyte develops, the nucleus is usually located in the cell's distal region, which is typically the future anterior of the embryo (Goldstein and Hird, 1996). In most oocytes (83%, $n = 60$), the nucleus showed periods of directed migration where it moved several micrometers to the oocyte's distal surface over an ~20-min period (Fig. 3b), the last migration occurring from approximately −31 to −12 min on average. This off-center placement of the nucleus is the only sign of polarization in the oocyte, and its significance is unclear. Oocytes in which the nucleus fails to reach the distal surface undergo normal embryogenesis (data not shown), and studies of fertilization suggest that the sperm entry point is the sole determinant of the anterior–posterior axis in *C. elegans* embryos (Goldstein and Hird, 1996). Late oogenesis is also characterized by large increases in both oocyte nuclear and oocyte cellular volumes. Nuclear volume increases from ~300 μm^3 at −140 min to ~700 μm^3 at −10 min, while cellular volume increases from ~12,000 to ~20,000 μm^3. As noted above, cellular volume increase im-

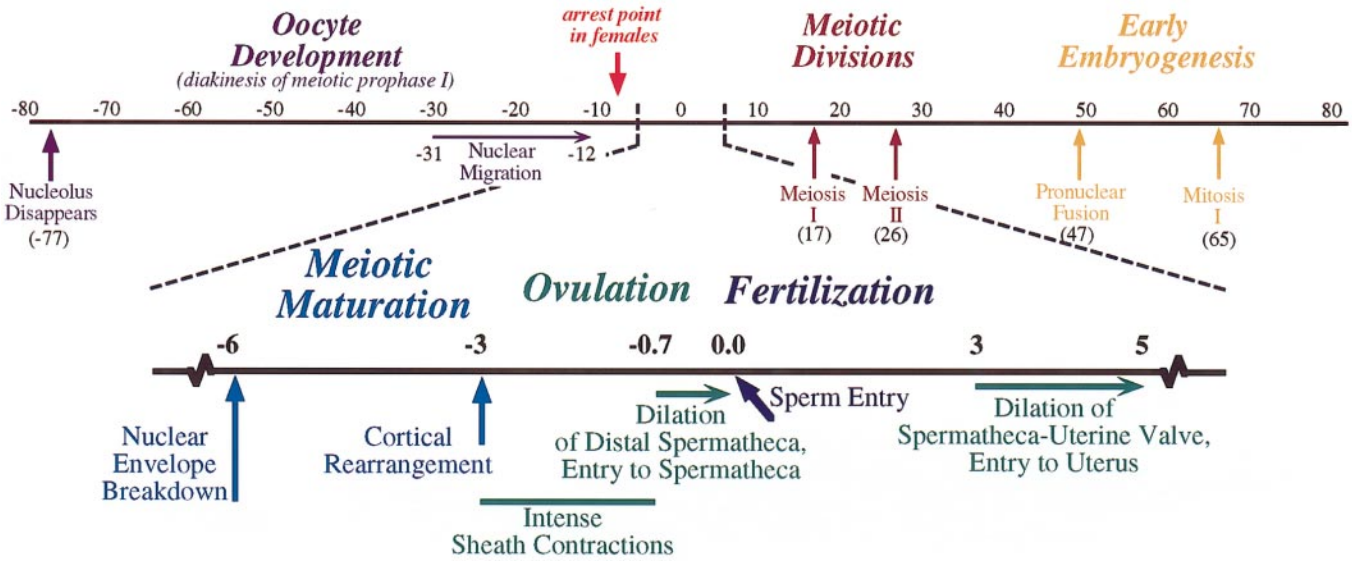


FIG. 2. Time line depicting the landmark morphological events of oocyte development, meiotic maturation, ovulation, fertilization, the meiotic divisions, and early embryogenesis for one oocyte. The end of ovulation (closing of the distal spermatheca) is defined as 0 min and coincides closely with fertilization. Events from −80 to +80 min are shown. The −6- to +6-min interval is expanded to show the closely coupled events of oocyte maturation and ovulation. The oocyte arrest point in females occurs after the events of late oogenesis but prior to the events of maturation and ovulation. However, its exact placement on the time line is arbitrary. See the text for further description, Table 2 for the data used to construct the time line including standard deviations, Fig. 3 for photographs of events, and Fig. 5a for a sheath activity profile.

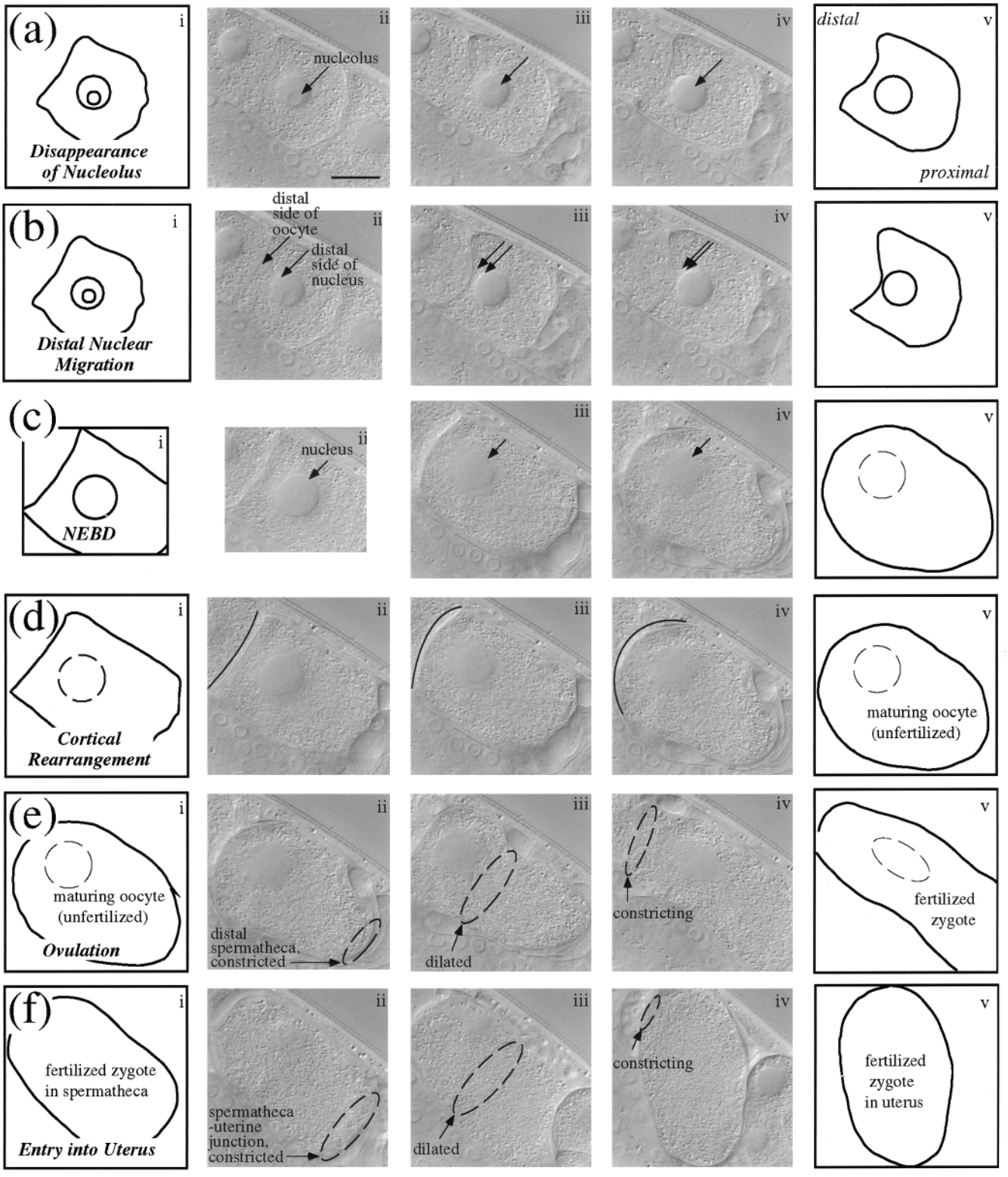
mediately prior to maturation is more pronounced in young adults that have recently switched from spermatogenesis to oogenesis since oocytes in older adults increase in size earlier in progression through the proximal gonad arm.

Oocyte meiotic maturation. In adult wild-type hermaphrodites, maturation and ovulation occurs every ~23 min in each arm and is limited to the most proximal oocyte. Maturation is characterized by two visible events occurring in quick succession within the oocyte. First, nuclear envelope breakdown (NEBD) begins at ~−6 min as the distinct edge which separates the nucleus from the cytoplasm begins to fade (Fig. 3c). By ~2 min postovulation, the nucleus is no longer recognizable. Second, cortical rearrangement begins at ~−3 min, rapidly transforming the oocyte from a cylindrical to ovoid shape (Fig. 3d). This oocyte shape change appears to be intrinsically driven and is not dependent on the contractions of the surrounding somatic sheath, since ablation of sheath cells does not interfere with cortical rearrangement (McCarter *et al.*, 1997). During meiotic maturation, chromosome arrangement also changes as the bivalents leave diakinesis of meiotic prophase and begin to align on the metaphase plate (Albertson, 1984; Albertson and Thomson, 1993). A time course for assembly of the meiosis I spindle has not been determined.

Oocyte ovulation. Ovulation is characterized by two processes that occur in the somatic cells: increasing contractile activity in the gonadal sheath cells and relaxation or dilation of the spermatheca. Prior to maturation and ovulation, the myoepithelial sheath cells of the gonad contract at a basal level of 10–13 contractions per minute. Following oocyte maturation, the rate of sheath contraction increases to ~19/min (Fig. 5a) and the contractions become increasingly vigorous. At −0.7 min, the distal spermatheca dilates and is pulled over the most proximal oocyte by the contracting sheath (Fig. 3e) so that by 0 min the oocyte has entered the lumen of the spermatheca and the distal spermatheca has closed behind the oocyte. Sperm entry at fertilization is difficult to visualize, but has been observed to occur immediately upon entry of the oocyte into the spermatheca (Ward and Carrel, 1979). Cytoplasmic streaming in the oocyte corresponds to the time of fertilization (but see Table 2, note f). From +3 to +5 min, the spermathecal-uterine valve dilates (Fig. 3f), and the fertilized egg moves into the uterus.

In the Absence of Sperm, C. elegans Oocytes Are Capable of Extended Cell Cycle Arrest in Diakinesis of Meiotic Prophase

In many species, oocytes enter an extended arrest in meiotic prophase before maturation (Masui and Clarke, 1979; Eppig, 1996). For human oocytes, this arrest can last for decades. In adult *C. elegans* hermaphrodites with sperm, where assembly-line-like production generates a maturing/ovulating oocyte approximately every 23 min, we believe that there is no arrest as the signal for maturation (sperm) is constitutive (see below, and Discussion). However, arrest in diakinesis is clearly observed when sperm are absent, as in



the "females" produced by null mutations in the sex determination genes *fem-1,2,3* and *fog-1,2,3* (Schedl, 1997). In mutant females, where all germ cells develop as oocytes and no sperm are introduced by mating, oocytes arrest in diakinesis for hours or even days. Nevertheless, the most proximal oocyte in female gonad arms do stochastically mature and ovulate at a very low rate (less than 1/40 the rate for hermaphrodites), perhaps reflecting an inability of *C. elegans* oocytes to maintain arrest indefinitely.

The diakinesis arrest point in females serves as a convenient marker for separating the events of late oogenesis from those of oocyte maturation and ovulation (Fig. 2). While maturation is impeded, the events of late oogenesis (i.e., cellularization, nucleolar disappearance, nuclear migration, cellular and nuclear volume increase, and progression through meiotic prophase up to diakinesis) occur without interruption in young adult females; arrested oocytes are of full size, lack visible nucleoli, and have large distally located nuclei with condensed chromosomes in diakinesis. Because oogenesis continues without oocytes exiting the gonad, a typical adult female can have 25 or more oocytes stacked in the gonad arm. While stochastic maturation results in the first oocyte in the gonad arm leaving its arrested state after ~14 h, the arrest can last for days in more distal oocytes. (The 10th oocyte, for example, can remain in diakinesis for >5 days.)

Spermatozoa, Spermatids, and Spermatocytes Promote Oocyte Meiotic Maturation

Mating virgin females with wild-type males induces previously arrested oocytes to begin maturation and ovulation, suggesting that spermatozoa promote oocyte meiotic maturation. To quantify these observations, we measured oocyte maturation rates in multiple situations where sperm were either present or absent. In all cases where wild-type sperm were present, oocytes matured at an average rate of >2 maturations per gonad arm per hour, including unmated and mated hermaphrodites, as well as mated females (Figs. 4a and 4b). In all cases where sperm were absent, oocytes matured at a very low rate of ≤ 0.1, including all unmated females and old "purged" hermaphrodites which had exhausted their supply of self-produced sperm (Fig. 4c). The act of mating without introduction of sperm does not trigger oocyte maturation; females mated with *glp-4(bn2)* males raised at 25°C, which have gonads lacking sperm (Beanan and Strome, 1992), did not show an increase in the maturation rate of oocytes (Fig. 4c). Therefore, in all cases, a high rate of oocyte maturation correlates with the presence of sperm.

During spermatogenesis, primary spermatocytes divide to form spermatids. Spermatids in turn are activated by contact with the spermatheca to become motile spermatozoa capable of fertilization (L'Hernault, 1997). To investigate whether sperm must be capable of fertilization to promote maturation, we examined mutants defective in the formation of spermatozoa. At 25°C, *fer-1(b232ts)* spermatozoa are motility defective with short pseudopods and *fer-3(hc3ts)* spermatids are incapable of differentiating into spermatozoa (Argon and Ward, 1980); neither type of sperm is capable of fertilizing oocytes. In *fer-1(b232ts)* and *fer-3(hc3ts)* hermaphrodites raised at 25°C, as well as in females mated with *fer-1(b232ts)* males raised at 25°C, the rate of oocyte maturation is comparable to the wild-type hermaphrodite rate (Fig. 4d), indicating that sperm can promote oocyte maturation without being capable of spermatozoa formation or fertilization. These observations agree with the finding that many sperm-defective mutants lay oocytes at a rate comparable to wild-type *C. elegans* hermaphrodites (L'Hernault *et al.*, 1988; Singson, *et al.*, 1998).

Interestingly, even developmentally arrested spermatocytes in the gonad arm can induce oocyte maturation. In *spe-4(q347)*, primary spermatocytes complete meiosis but fail to undergo cytokinesis to form spermatids (L'Hernault and Arduengo, 1992). *spe-4(q347)* young adult hermaphrodites show a rate of oocyte maturation similar to that observed in wild-type young adult hermaphrodites (1.2 ± 0.3 vs 1.3 ± 0.3) (Fig. 4d). The resulting ovulations deposit the *spe-4* defective spermatocytes into the uterus where

FIG. 3. Nomarski micrographs of one oocyte in a young adult over 1.5 h of its development. Each row of photographs depicts the completion of one event (ii, before; iii, during; iv, after). i and v are tracings of ii and iv, respectively, outlining the oocyte's features before and after the event. Proximal, lower right corner of each photograph. Ventral surface of worm, upper right corner of each photograph. The oocyte begins in the second most proximal position in a, ii and b, ii and occupies the most proximal position from a, iii to e, ii. [a, i–v] Disappearance of nucleolus. The nucleolus (arrow) present in ii has faded from view by iv. [b, i–v] Distal migration of the nucleus. The nucleus, ~7 μm from the distal surface of the oocyte in ii (arrows), has moved to within ~1 μm by iv. The oocyte surface has become concave (see text). [c, i–v] Beginning of nuclear envelope breakdown (NEBD). The edge of the nucleus (arrow) which is distinct in ii has faded from view by iv. The nucleoplasm remains visible until ~2 min after ovulation. [d, i–v] Cortical rearrangement. The oocyte changes from a cylindrical to ovoid shape (i.e., from a rectangular to elliptical shape in the cross section of the photo). Note the change of the oocyte's distal surface (line) from concave in ii to convex in iv. [e, i–v] Ovulation. The distal spermatheca (dotted line) begins constricted and proximal to the oocyte in ii, dilates and is pulled over the oocyte by the contracting sheath cells in iii, and ends reconstricted and distal to the oocyte in iv. Fertilization of the oocyte apparently occurs during e, iii to iv, forming the zygote. [f, i–v] The spermatheca–uterine junction begins constricted and proximal to the zygote in ii, dilates and moves over the zygote in iii, and ends reconstricted and distal to the zygote in iv. Egg shell formation, which maintains the zygote in the shape established by cortical rearrangement, begins after fertilization and is completed while the zygote is in the uterus (Ward and Carrel, 1979; Strome, 1986a). Scale bar, 10 μm.

TABLE 2
Time of Events in Oocyte Development, Maturation, and Ovulation

Developmental stage	Event	Time of event[a] (min)	n[b]
Oocyte development	Nucleolus disappears[c]	−77 ± 28	24
	Begin last nuclear migration	−31 ± 9	10
	End last nuclear migration[d,e]	−12 ± 6	10
Meiotic maturation	Begin nuclear envelope breakdown	−6 ± 2	60
	Begin cortical rearrangement[f]	−3 ± 2	60
Ovulation	Begin entry into spermatheca[g]	−0.7 ± 0.2	60
	End entry into spermatheca, and fertilization[h]	0 (by definition)	60
	Begin entry to uterus	3 ± 1	60
	End entry to uterus	5 ± 1	60
Meiotic divisions	Meiosis I, polar body 1[i]	17 ± 3	4
	Meiosis II, polar body 2	26 ± 4	4
Embryogenesis	Pronuclear fusion	47 ± 13	7
	Mitosis I	65 ± 17	7

[a] Times are rounded up to the nearest minute.

[b] n, the number of oocytes surveyed. Oocytes from both adults and young adults are included. The timing of these events is similar in both adults and young adults (data not shown).

[c] The nucleolus takes 10–15 min to fade from view. Nucleolar disappearance has been described by electron microscopy (Abi-Rached and Brun, 1978).

[d] In addition to the nucleus moving distally, the distal surface of the oocyte can also bend to meet the nucleus (Fig. 3b), suggesting a physical connection under tension. The physical movement of the gonad during ovulation of preceding oocytes often disrupts the distal positioning of nuclei in subsequent oocytes. A single nucleus can be observed to lose its distal position and remigrate distally up to three times during late oogenesis, each migration occurring between the ovulations of preceding oocytes (data not shown).

[e] In most cases (75%, $n = 44$) the nucleus is centered along the oocyte's dorsal–ventral axis (i.e., the sides of the nucleus are 7–8 μm from both the dorsal or ventral surface of the oocyte). In the remaining 25% of cases the nucleus is off-center with one side of the nucleus only 2–6 μm from either the dorsal or ventral surface. Oocytes with nuclei displaced to the dorsal or ventral side showed no defect in maturation, ovulation, or embryogenesis.

[f] Cortical rearrangement has several potential roles. It may allow the first oocyte to separate from the gonadal syncytium, prevent tearing of the oocyte as it is deformed during ovulation, or serve as the template to maintain the embryo in an ovoid shape until the egg shell is laid down (Wharton, 1983). Cortical changes at maturation have been investigated in starfish oocytes where they entail formation of actin spikes extending from the cortex (Otto and Schroeder, 1984), and disassembly of cortical microtubules and intermediate filaments (Schroeder and Otto, 1991).

[g] Spermatids remain in the proximal gonad arm of the hermaphorodite until ovulations begin. The very first ovulation, therefore, differs from subsequent ovulations in that the oocyte must act as a "plunger" to move a large number of spermatids into the spermatheca. Subsequent ovulations move smaller numbers of sperm. Despite the larger volume which the first ovulation must move, it occurs equally rapidly to later ovulations (interval, 0.7 ± 0.2 min; $n = 7$).

[h] During oocyte development (prior to −6 min) and maturation (−6 to 0 min), little movement is observed in the oocyte cytoplasm. Upon spermathecal entry at 0 min, oocyte cytoplasmic granules begin circular streaming. While cytoplasmic streaming was initially thought to indicate fertilization of the oocyte (Ward and Carrel, 1979; Kimble and Ward, 1988), we observe streaming in ovulated oocytes which are not fertilized, including ovulated oocytes of *fer-1*(*hc13* and *b232*) and *spe-4(q347)* hermaphrodites where sperm are incapable of fertilization, ovulated oocytes of *fog-2(q71)* females mated with *fer-1(hc13* or *b232)* males, and rare ovulated oocytes of *fem-3(e1996)* and *fog-3(q443)* females where no sperm are present. Further, streaming is also observed in mature oocytes of the mutants *lin-3(n1058)* and *let-23(sy10)* where ovulation is defective so that the oocyte never reaches the spermatheca (J. McCarter, B. Bartlett, T. Dang, R. J. Hill, M. Lee, and T. Schedl, in preparation, 1998). These observations indicate that oocyte cytoplasmic streaming requires only oocyte maturation and is not dependent on ovulation or fertilization.

[i] Meiosis I and II were timed by the earliest visualization of polar body 1 and polar body 2, respectively (also see Kemphues *et al.*, 1986).

they are degraded. In older *spe-4(q347)* adult hermaphrodites, oocyte maturations are rare, similar to the situation in females and consistent with the elimination of *spe-4* mutant spermatocytes. Taken together, these results indicate that there is a factor from spermatozoa, spermatids, and spermatocytes (and possibly earlier stages) that can promote maturation of oocytes in *C. elegans*.

Sheath Contractile Activity and Spermathecal Dilation during Ovulation Follow a Reproducible Sequence Which Requires Maturing Oocytes

Gonadal sheath contractile activity and distal spermathecal dilation, in hermaphrodites, undergo a reproducible series of changes during ovulation (Fig. 5a). Following

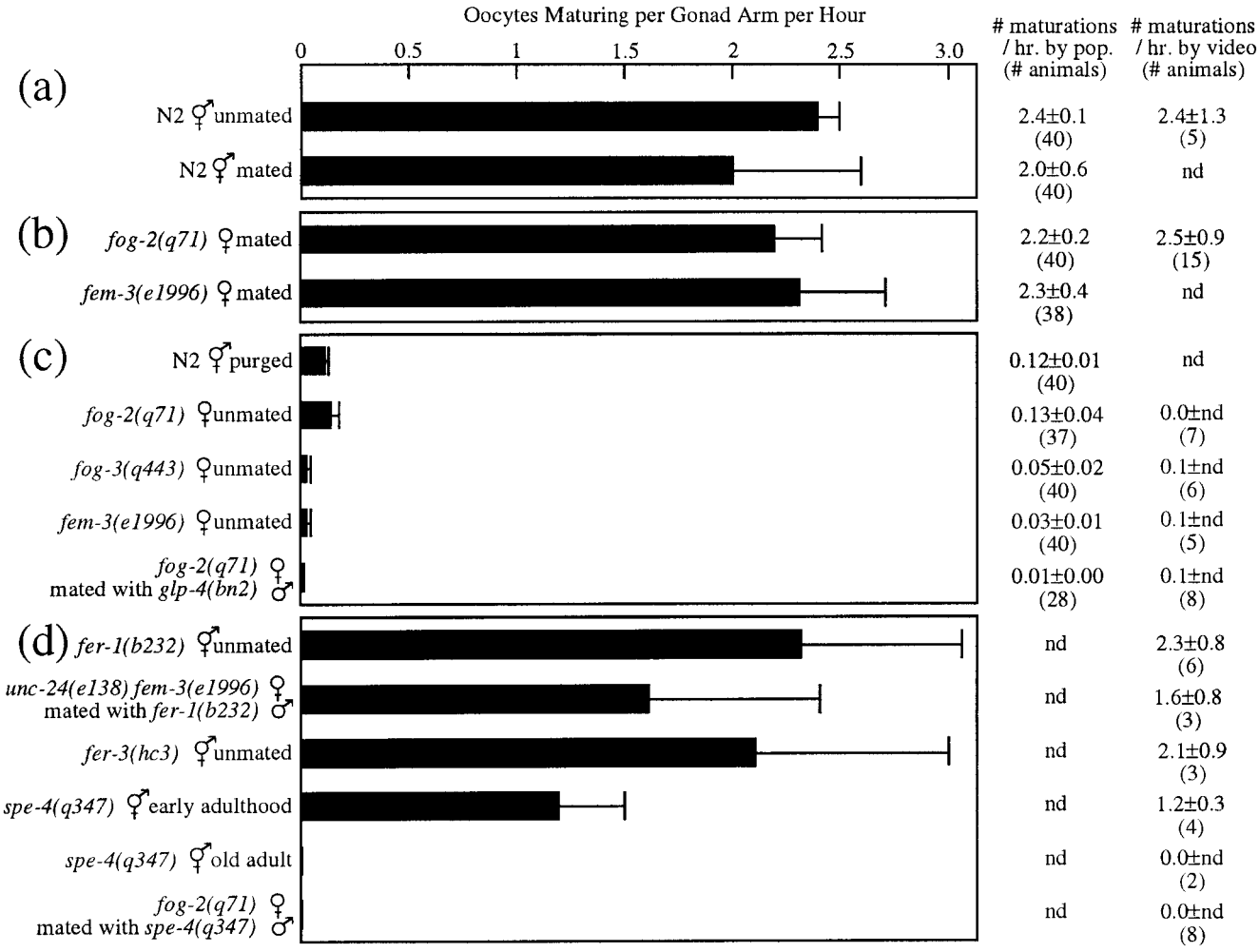


FIG. 4. Oocyte maturation rates. Rates were determined by two methods: measuring total embryo production in populations at 20°C (see Materials and Methods), and by observation of maturation/ovulation with time-lapse microscopy. In a, b, and c, rates from population samples are graphed whereas in d, rates from microscopy are displayed. Error bars indicate standard deviations. nd, not determined. # animals, the number of animals surveyed by each method. (a) Wild-type (N2) hermaphrodites. (b) Mated females. Maturation rates are similar to those observed in adult hermaphrodites. (c) Animals lacking sperm, including unmated females. Oocyte maturations are rare. (d) Mutants with defective sperm. Rates were determined exclusively by time-lapse microscopy since large numbers of unfertilized oocytes in populations cannot be counted with accuracy. *fer-1(b232ts)* and *fer-3(hc3ts)* animals were raised at 25°C. The *fer-1(b232ts)* strain contains *him-5(e1490)* which has no effect on the maturation rate. The maturation rate observed in *unc-24(e138) fem-3(e1996)* females mated with *fer-1(b232ts)* males is not significantly different from the rate in *unc-24(e138)* hermaphrodites alone, which is lower than wild-type (data not shown).

oocyte maturation, the rate of sheath contraction increases (peaking at ~19/min) and the contractions become more vigorous (i.e., show increased displacement). As the spermatheca dilates and then reconstricts at ovulation, the sheath appears to tonically (continuously) contract and then relax. Its contractile rate drops precipitously (to ~9/min) and takes several minutes to recover. This cycle is repeated at the next ovulation. The same pattern is observed in mated females (Fig. 5b) and in mated hermaphrodites (data not shown).

Cyclical changes in sheath activity and spermathecal relaxation are observed only in the presence of maturing oocytes. Sheath activity first becomes detectable in mid-L4 animals, and increases as animals enter adulthood (Fig. 6), yet none of these activity traces show cyclic changes (data not shown). In all cases where maturing oocytes are absent, including unmated females (Fig. 5c), animals with masculinized germlines containing only spermatids (Fig. 5d), and unmated and mated animals with tumorous germlines (data not shown), sheath con-

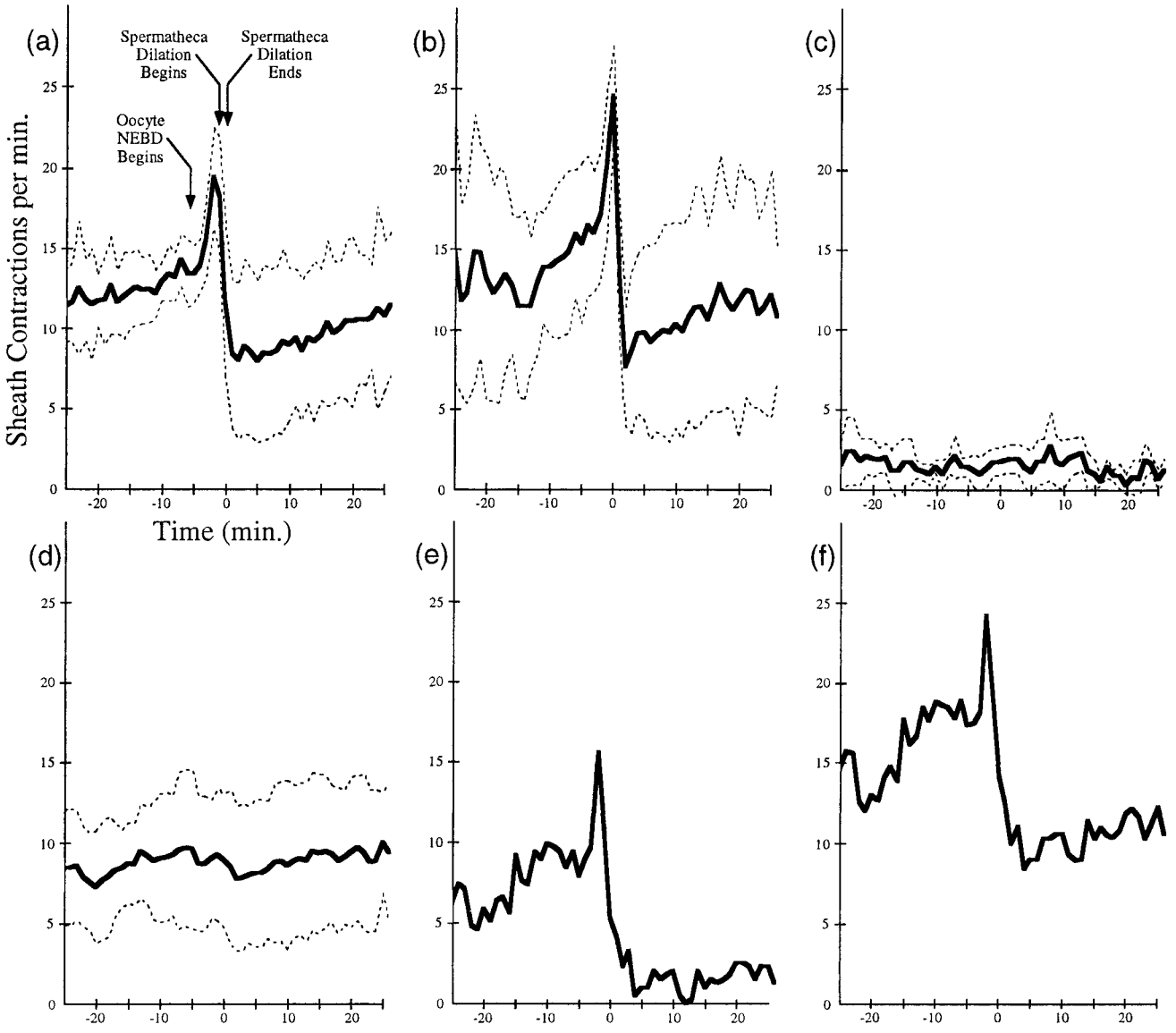


FIG. 5. Sheath contractile activity profiles. Number of sheath contractions per minute is shown over time. Thick line, average. Dotted line, standard deviation. (a) Wild-type (N2) adult hermaphrodite, one ovulation cycle, $n = 10$. Profiles are synchronized at the end of ovulation (zero min). (b) Mated *fog-2(q71)* female, one ovulation cycle, $n = 15$. Profiles are synchronized at the end of ovulation. (c) Unmated *fog-2(q71)* female, $n = 4$. No ovulations occurred. Profiles show no dramatic temporal trend and are randomly overlaid. (d) *fem-3(q20gf)* hermaphrodites, grown at 25°C, with a masculinized germline (i.e., vast excess of spermatids and no oocytes), $n = 6$. Contractile activity is significantly increased compared to females, but profiles show no dramatic temporal trend and are randomly overlaid. (e) Unmated females during rare instances of maturation/ovulation, $n = 2$. Profiles are synchronized at the end of ovulation. (f) A composite profile showing the addition of sheath activity from sperm alone (d) and maturing oocytes alone (e) creating a resulting trace similar to that of an ovulating adult with both sperm and maturing oocytes. Maturation and ovulation was also observed in the hermaphrodite nematode *Caenorhabditis briggsae;* sheath activity during the ovulation cycle was similar to that observed in the *C. elegans* hermaphrodite (data not shown, $n = 2$). Control recordings were also made of anesthetized *C. elegans* males. Males lack gonadal sheath cells and contractions like those seen in hermaphrodite gonad arms were not observed (data not shown, $n = 4$).

tractile activity traces are relatively constant without cyclic increases or decreases. Similarly, none of these conditions cause spermathecal dilation.

Sperm are not required for either sharp increases in sheath activity or spermathecal dilation. In two rare cases where oocyte maturation/ovulation was observed in unmated fe-

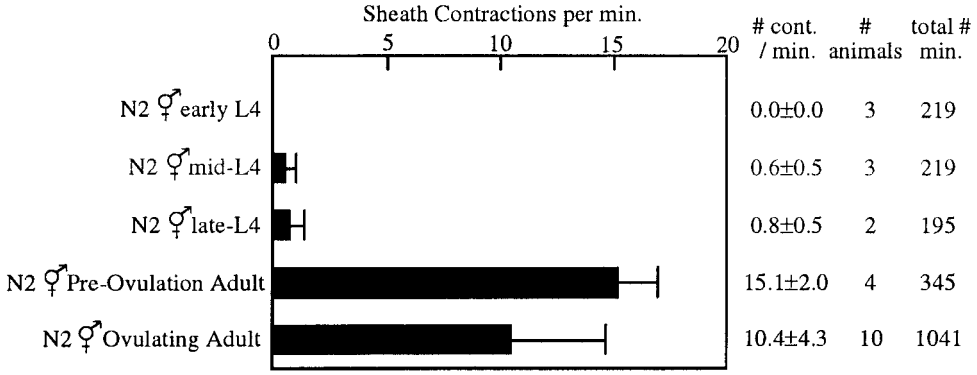


FIG. 6. Average levels of sheath activity in developing wild-type (N2) hermaphrodites. Error bars indicate standard deviations. # animals, the number of animals of each age surveyed. Total # min, the number of minutes surveyed. Sheath contractions are first observed in mid-L4, corresponding to our first detection of actin fibers in proximal sheath cells (McCarter *et al.*, 1997) and the onset of spermatogenesis. (Sheath activity is therefore established before oogenesis begins at the L4/adult molt.) Average levels of sheath activity are higher in young adults (preovulation) than in ovulating adults because the sheath in young adults contracts at a high steady rate without the trough in activity that follows each ovulation (Fig. 5a).

males by video microscopy, sheath activity increased and the spermatheca dilated as the oocyte was ovulated (Fig. 5e). These findings indicate that the maturing oocyte triggers both the rise in sheath contractile activity and the dilation of the spermatheca that occurs at ovulation.

Spermatozoa and Spermatids Promote Sheath Contractile Activity in the Absence of Oocytes

In unmated females, where neither sperm nor maturing oocytes are present, sheath activity is ~1.5 contractions/min, or one-seventh the average hermaphrodite rate (compare Figs. 5a to 5c and 7a to 7d). Mating females with wild-type males, which introduces sperm and causes oocytes to mature, restores wild-type levels of sheath activity (Figs. 5b and 7b). Animals with fertilization-defective sperm also display wild-type levels of sheath activity (Fig. 7c). The act of mating alone without sperm does not trigger sheath activity since mating females with *glp-4(bn2)* males did not cause increased sheath activity (Fig. 7d). Do sperm cause the sheath to contract via their effect on oocyte maturation, or can sperm directly trigger sheath activity independent of oocytes? Surprisingly, we find that spermatids can stimulate the sheath to contract at 10–14 contractions/min in the absence of oocytes. This steady-state rate, similar to that observed in hermaphrodites between ovulations, is observed in masculinized germ lines, such as *fem-3(q20)* (Barton *et al.*, 1987) and *gld-1(oz10)* (Francis *et al.*, 1995), which contain only spermatids and no oocytes in the gonad arm (Figs. 5d and 7e).

We further tested the effect of sperm on sheath contraction in the absence of oocytes by measuring sheath contraction rates in unmated and mated animals with tumorous germlines. Unmated animals with tumorous *gld-1(q485) fog-3(q443)* germlines (Francis *et al.*, 1995; Ellis and Kimble, 1995) containing neither sperm nor oocytes show low levels of sheath activity. Introducing spermatozoa by mating resulted in high levels of sheath activity in all cases (Fig. 8). Therefore, spermatids present in the gonad arm, as well as spermatozoa introduced into the spermatheca by mating, induce sheath activity in the absence of oocytes.

In summary, both sperm and oocytes have effects on the somatic gonad during ovulation; sperm generate a steady-state rate of sheath activity, while the maturing oocyte both generates an increase in sheath activity and induces spermathecal relaxation resulting in oocyte ovulation. Interestingly, the effect of sperm and maturing oocytes on sheath contractile rates appears to be additive. When a trace of sheath activity from sperm alone (Mog hermaphrodite, Fig. 5d) is added to a trace of sheath activity from maturing oocytes alone (female, Fig. 5e), the resulting trace (Fig. 5f) is indistinguishable from that of an ovulating adult with both sperm and maturating oocytes (Figs. 5a and 5b).

Additional Factors Affect Oocyte Meiotic Maturation Including Distal–Proximal Position of the Oocyte

Factors in addition to the presence of sperm play roles in the regulation of oocyte maturation. In wild-type hermaphrodites, maturation is never observed to occur out of proximal-to-distal order; within the single row of oocytes in the gonad arm only the most proximal oocyte matures ($n = 60$). Because ablation of the most proximal four sheath cells in wild-type hermaphrodites delays maturation (McCarter *et al.*, 1997), we considered the hypothesis that direct contact with proximal sheath cells (fourth and fifth sheath pair (Kimble and Hirsh, 1979)) might promote maturation. Proximal sheath are connected to oocytes via gap junctions which would allow for direct cytoplasmic communication (Rose *et al.*, 1997). How-

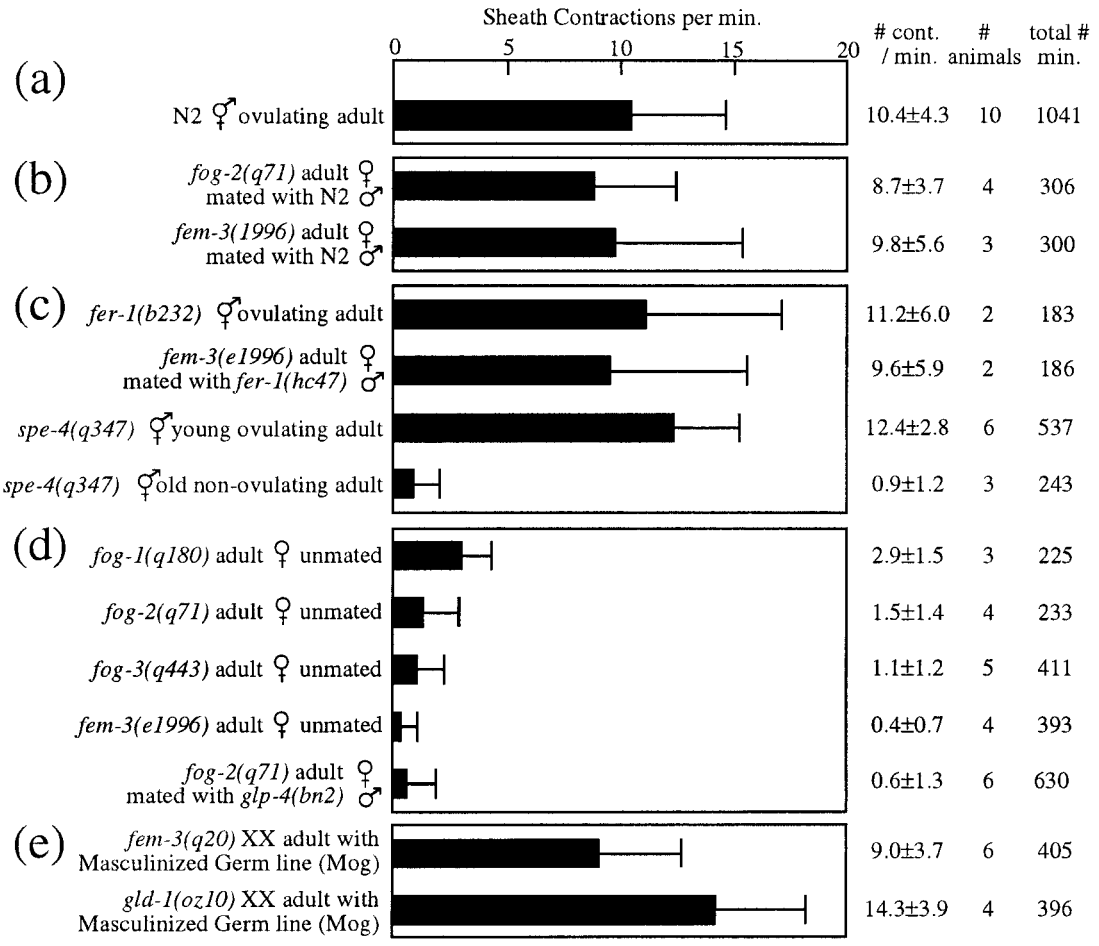


FIG. 7. Average levels of sheath activity in *C. elegans* wild-type hermaphrodites and mutants. Error bars indicate standard deviations. # animals, the number of animals of each genome surveyed. Total # min, the number of minutes surveyed. (a) Wild-type (N2) adult hermaphrodite. (b) Mated females. (c) Hermaphrodites with defective sperm and females mated to males with defective sperm. The *fer-1(b232ts)* strain contains *him-5(e1490)* which has no effect on sheath activity during ovulation. (d) Unmated females. Both *fog-1(q180)* and *fog-3(q443)* female strains carried *unc-13(e51)* which has no effect on sheath activity during ovulation ($n = 3$). (e) Animals with masculinized germlines. *fem-3(q20gf)* animals were maintained at 25°C where their germlines are fully masculinized (Barton *et al.*, 1987). The *gld-1(oz10)* strain included *unc-13(e51).*

ever, several observations suggest that direct contact with the most proximal sheath cells is not required for maturation. In *fem-3(q20)* animals raised at 15–20°C excess sperm are produced which fill the proximal gonad arm so that oogenesis is displaced distally. Oocytes in *fem-3(q20)* animals with excess sperm still mature in proximal-to-distal order, but maturation often begins while the first oocyte is in the loop of the gonad arm. (The gonad arm loop is enclosed by the second distalmost pair of the five sheath cell pairs in the arm.) We have also observed oocytes maturing in the loop of the gonad arm in mutants with the Emo phenotype. In *emo-1(oz1)* (Iwasaki *et al.*, 1996), *lin-3(n1058),* and *let-23(sy10)* (J. McCarter, B. Bartlett, T. Dang, R. J. Hill, M. Lee, and T. Schedl., in preparation, 1998), where ovulation is defective and mature oocytes are trapped in the arm, oocytes can mature in proximal-to-distal order without movement proximally so that oocytes in the loop eventually mature. Therefore, direct contact with the most proximal sheath cells is not required for maturation.

DISCUSSION

Our analysis of the temporal coupling of maturation and ovulation in *C. elegans* demonstrates that cell cycle progression in one cell (oocyte meiotic maturation) can stimulate neighboring cells to carry out coordinated motor activities (sheath contraction and spermathecal dilation). Further, regulation of these motor activities likely occurs without direction from the nervous system since none of the cells involved are innervated (White *et al.*, 1986). The evidence presented here suggests a system of regulation which includes germ cell to germ cell and germ cell to

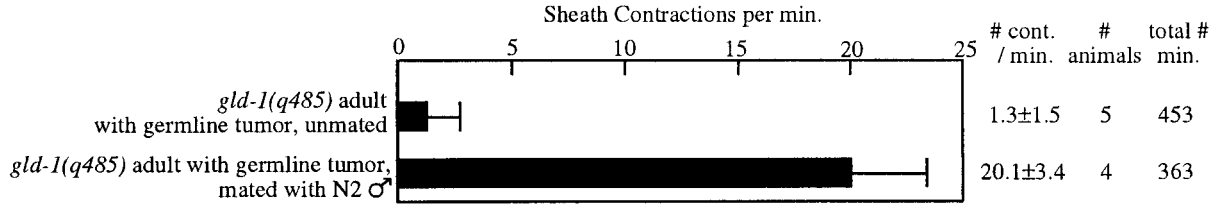


FIG. 8. Average levels of sheath activity in adults of the *unc-13(e51)gld-1(q485)fog-3(q443)* tumorous strain, unmated and mated to wild-type males. Filled bars give averages and error bars give standard deviations. (*fog-3(q443)* was included to eliminate the spermatogenesis that can occur in *q485* in an *unc-13* background (Francis *et al.*, 1995).) Data include only animals early in adulthood. In mated *gld-1(q485)* animals, sheath activity was steady without peaks and troughs.

somatic cell signaling. First, oocyte maturation is induced by sperm (i.e., spermatocytes, spermatids, spermatozoa) temporally and spatially independent of the sperm's role at fertilization (Fig. 9a). Second, sperm also promote steady-state sheath contractile activity with or without oocytes (Fig. 9b). Third, the maturing oocyte modulates sheath activity during ovulation (Fig. 9c). Fourth, the maturing oocyte induces spermathecal relaxation during ovulation (Fig. 9c).

Regulation of Oocyte Meiotic Maturation by Sperm

Stimulation of oocyte production by sperm in *C. elegans* has been recognized previously (Nelson *et al.*, 1978; Ward and Carrel, 1979; L'Hernault *et al.*, 1988; Singson *et al.*, 1998). Our findings show that oocyte meiotic maturation (NEBD and cortical rearrangement) is the regulatory point upon which sperm act. The biochemical mechanism by which sperm promote oocyte maturation is unknown. Our studies of oocyte maturation in mutants with defective spermatogenesis help to establish some of the characteristics of a putative sperm-derived factor promoting oocyte maturation. First, the factor functions independently of the interaction between sperm and oocytes during fertilization since fertilization-defective sperm are still capable of promoting oocyte maturation. Second, the factor is found in spermatozoa, spermatids, and *spe-4* mutant spermatocytes, indicating that it is produced during spermatogenesis. Third, the factor may be secreted by sperm and act at a distance since sperm transferred from males to females and residing in the spermatheca cause oocyte maturation in the gonad arm. However, we have not addressed whether sperm might directly contact the oocyte through the distal spermathecal constriction nor whether the signal might be indirectly conveyed via the spermatheca or sheath. *C. elegans* sperm can be separated from worm carcasses for biochemical fractionation (Klass and Hirsh, 1981) and can also be injected into the uterus for *in vitro* fertilization (LaMunyon and Ward, 1994). Such techniques may allow purification of a sperm-produced factor which induces oocyte maturation. In *Drosophila,* a 36-amino-acid peptide has been isolated from male seminal fluid which greatly stimulates ovulation; however, the effect of this peptide on oocyte maturation, if any, is not known (Chen *et al.*, 1988).

Additional Determinants Affecting Oocyte Meiotic Maturation

Oocyte maturation is regulated in other ways. First, maturation occurs only in the most proximal oocyte in the gonad arm (Fig. 1), even for the rare maturations occurring in the absence of sperm. This restriction suggests a role for proximal–distal position in maturation. The somatic gonadal sheath cells promote maturation (McCarter *et al.*, 1997; Rose *et al.*, 1998). However, direct contact of the oocyte with the most proximal sheath cells (fourth and fifth pair, see Kimble and Hirsh, 1979) is not necessary for maturation; proximal-to-distal maturation occurs normally in mutants where the most proximal oocyte resides in the loop region (see Results). One possibility is that the proximal gonad produces a diffusible signal. The most proximal oocyte would be exposed to a higher concentration of this signal than more distal oocytes. Signaling within the germline is also likely to be an important part of the restriction mechanism. The most proximal oocyte is unique in that there are no oocytes proximally while oocytes in the second and more distal positions have oocytes both proximally and distally. The proximal oocyte might produce a signal that inhibits maturation of distal oocytes. Additionally, the most proximal oocyte may escape from an inhibitory signal from distal oocytes. Cytological data have recently supported the view that the most proximal oocyte is distinct from more distal oocytes; the *air-1* protein preferentially localizes to chromosomes in the most proximal oocyte while it is primarily distributed in the cytoplasm of more distal oocytes (J. Schumacher, A. Golden, and P. Donovan, personal communication).

Second, maturation occurs only in an oocyte which has completed the events of oocyte development, including nucleolus breakdown, distal nuclear positioning, meiotic prophase progression/chromosome condensation, and expansion of oocyte cellular and nuclear volume. Either dependent pathways or independent but coordinately timed pathways might ensure that the events of oocyte develop-

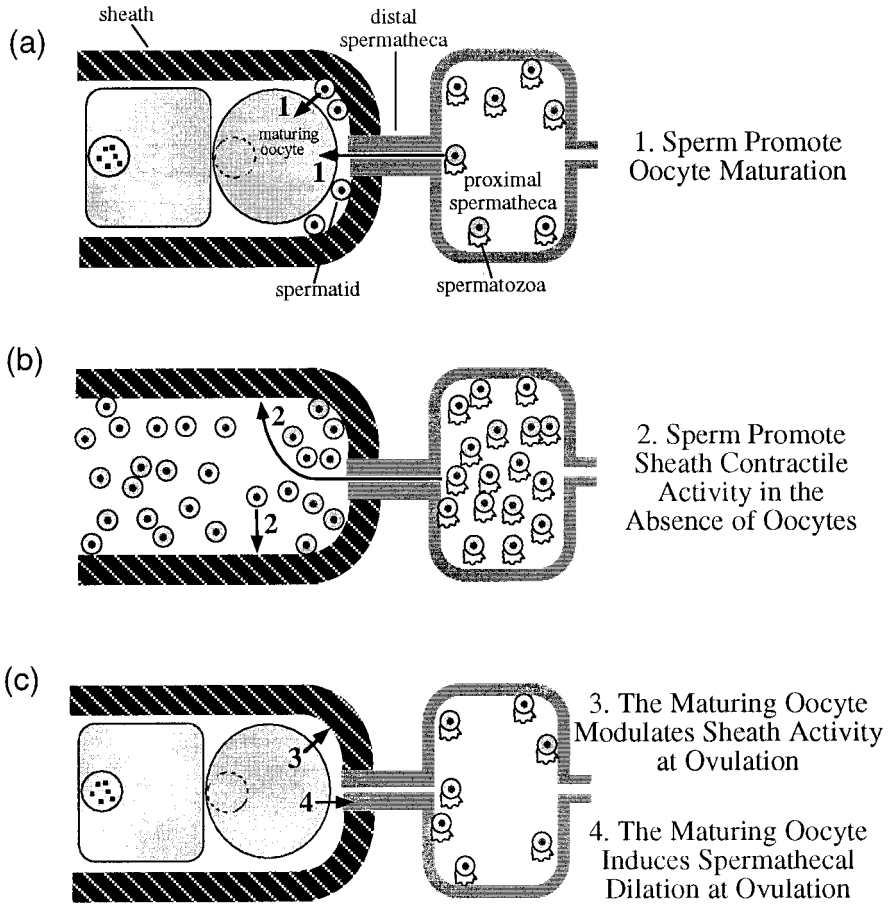


FIG. 9. The role of germ cell signaling in maturation and ovulation. (a) Sperm promote oocyte maturation. In the absence of sperm, oocytes enter prolonged arrest in diakinesis and only rarely mature. (b) Sperm promote sheath contractile activity in the absence of oocytes. This is true for spermatids in the gonad arm [*fem-3(q20gf)*] or spermatozoa introduced into the spermatheca of tumorous mutants by mating [*gld-1(q485)* hermaphrodite × wild-type male]. (c) The maturing oocyte modulates sheath activity and induces spermathecal dilation at ovulation. Following ovulation, sheath activity decreases to the lowest level in the cycle (see Fig. 5). Possibly, spermathecal reconstriction, or the zygote in the spermatheca, feeds back on the sheath leading to the observed decrease in activity following ovulation.

ment have been completed before allowing oocyte maturation. The dependence relationships of events within *C. elegans* oocyte maturation are currently undefined. For example, we do not know whether NEBD can occur in the absence of cortical rearrangement. Experiments suggest that there are independent pathways for mouse oocyte maturation where the cdc2/cyclin B complex (MPF) is needed for NEBD, and Mos and MAP kinase regulate microtubule and chromatin behavior (Verlhac *et al.*, 1996). Future studies may allow organization of the events of *C. elegans* oogenesis and meiotic maturation into pathways as has been accomplished for mitotic cell cycle events (Hartwell *et al.*, 1974; Murray, 1992).

Regulation of the Sheath and Spermatheca Myoepithelial Activities at Ovulation

Ovulation in *C. elegans* consists of a highly reproducible series of myoepithelial activities, each change presumably caused by altered ion channel activity within the cells. These activities include an increase in sheath contraction rate and intensity and dilation of the spermatheca leading to oocyte exit from the gonad arm, followed by a decrease in sheath contraction rate and intensity and reconstriction of the spermatheca. Although the sheath and spermatheca lack innervation (White *et al.*, 1986), a rather complex motor pattern is still assembled through sperm to sheath signaling for baseline contractile activity (Fig. 9b) followed by maturing oocyte to sheath and spermatheca signaling for contraction and dilation, respectively (Fig. 9c). These non-neuronally mediated contractile activities are similar in some respects to *C. elegans* muscular activity for feeding since pharyngeal pumping can still occur following the ablation of the entire pharyngeal nervous system (Avery and Horvitz, 1989).

Cell cycle progression is known to lead to changes in electrophysiological properties within the cycling cell (Day *et*

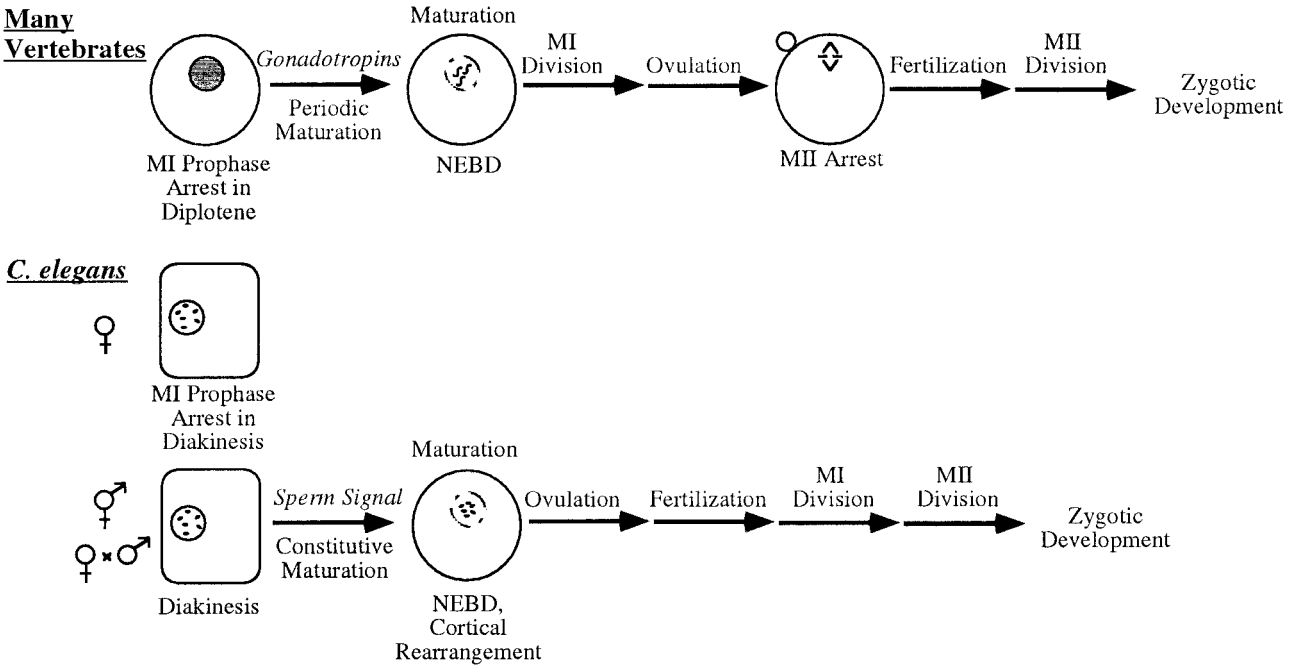


FIG. 10. Variations in oocyte meiotic progression between species. The progression of events from late prophase of oogenesis to the initiation of zygotic development is shown schematically for vertebrates and *C. elegans*. See text for details. Most vertebrates have meiotic arrest points in both prophase of meiosis I (oocyte) and at metaphase of meiosis II (unfertilized egg). For vertebrates, meiosis II arrest allows ovulation to be separated temporally and spatially from fertilization. *C. elegans* oocytes are capable of arrest in prophase of meiosis I (females) but in the presence of sperm progress directly to maturation. Since ovulation is immediately followed by fertilization in the spermatheca, a meiosis II arrest is unnecessary. Vertebrate oocytes arrest in the transcriptionally active diplotene stage while oocytes in *C. elegans* females arrest later, in diakinesis. The later arrest in *Caenorhabditis elegans* may allow a rapid transition to egg production (maturation, ovulation, and fertilization) following chance mating of a female or "purged" hermaphrodite with a male.

al., 1993). For instance, MPF activity during *Xenopus* oocyte maturation can inactivate a delayed rectifier potassium channel (Bruggemann *et al.,* 1997). Ovulation in *C. elegans* appears to provide a rare tractable example where cell cycle progression in one cell (the oocyte) leads to changes in contractile activity of neighboring cells (the sheath and spermatheca), presumably due to alteration of their electrophysiological properties. Recent investigations have begun to address the molecular mechanisms by which maturation and ovulation are coupled in *C. elegans.* We have demonstrated a role for the EGF-like ligand LIN-3 and the receptor tyrosine kinase LET-23 in oocyte signaling for spermathecal dilation (J. McCarter, B. Bartlett, T. Dang, R. Hill, M. Lee, and T. Schedl, in preparation). Clandinin and colleagues have identified two gene products, *lfe-1* and *lfe-2,* that likely act in LET-23 signal transduction to alter calcium levels within the spermatheca (Clandinin *et al.,* 1998).

Variations in Meiotic Progression and Comparative Reproductive Biology

The progression from oocyte to cleaving embryo involves a similar set of events in most organisms: meiotic maturation, the reductional (MI) and equational (MII) meiotic divisions, ovulation, and fertilization. However, the order of events and the position or utilization of control points can differ (Fig. 10). These differences presumably reflect adaptations to suit each species' reproductive biology. *C. elegans* is a species with a short life span and a life strategy based on producing a large number of progeny in as short a time as possible (Hodgkin and Barnes, 1991). Because of the worms' tube-shaped morphology, it produces oocytes in a single-file assembly-line-like fashion, where only one oocyte within a gonad arm can undergo maturation, ovulation, and fertilization at a time.

For many species, oocytes can undergo a prolonged arrest in prophase of meiosis I (Masui and Clarke, 1979). For *C. elegans,* oocytes arrest in diakinesis of prophase I in the absence of sperm. In vertebrates, oocyte arrest is relieved in a periodic manner, seasonally in many amphibians, by the estrus cycle in many mammals, and by the menstrual cycle in primates. In contrast, oocytes in *C. elegans* hermaphrodites with abundant sperm progress through prophase and mature in an assembly-line-like manner. There is no obvious diakinesis prophase arrest, consistent with a life strategy of producing a large number of progeny in a short time.

In many vertebrates, maturation/ovulation is temporally and spatially separated from fertilization. Following maturation (NEBD) and MI in the ovary, oocytes are ovulated and the eggs arrest at a second point, metaphase of MII, until fertilization. By contrast, for *C. elegans*, internal fertilization in the spermatheca results in a temporal and spatial coupling of maturation/ovulation and fertilization. There is no MII, postovulation, arrest. In the absence of fertilization, ovulated oocytes fail to complete meiosis, do not form an egg shell, and are removed from the reproductive tract by the egg-laying system (Ward and Miwa, 1978).

Selective Advantage in the Regulation of Maturation by Sperm

Why does a species with self-fertile hermaphrodites like *C. elegans* use a sperm-derived factor to stimulate oocyte maturation? One possibility is that the sperm dependence of oocyte maturation helps conserve resources following depletion of self-sperm in anticipation of later mating. Unmated wild-type hermaphrodites produce an average of 320 progeny, corresponding to the number of self-produced sperm, whereas mated hermaphrodites can generate as many as 1400 (Kimble and Ward, 1988). Since males are rare (Hodgkin *et al.*, 1979), hermaphrodites may travel for days or a lifetime without mating. Therefore, hermaphrodites depleted of self-sperm may greatly slow their rate of oocyte maturation to save valuable oocytes within the gonad arm. In their conservation of oocytes, hermaphrodites depleted of self-sperm are behaving like females of related nematode species like *C. remanei* which also retain their oocytes until sperm arrive (our unpublished observations). The sperm dependence of oocyte maturation may be a mechanism inherited from a male/female ancestor species and maintained in the hermaphrodite to take advantage of opportunities for cross-progeny production from mating.

ACKNOWLEDGMENTS

Strains, reagents, experimental protocols, and productive suggestions were provided by Leon Avery, Laura Wilson Berry, Ross Francis, Bob Goldstein, David Greenstein, Steve L'Hernault, Eric Lambie, Ann Rose, Bob Waterston, Shelly Weiss, Jim Waddle, and Sam Ward. We thank Eric Lambie, David Greenstein, and Yen Bui for their helpful comments. This research was supported by National Science Foundation Grant 9506220 to T.S. During a portion of this work, T.D. was supported by the National Science Foundation summer undergraduate research program in developmental biology (BIR-9531558) and B.B. received support from a Howard Hughes Medical Institute Grant to the Young Scientist Program for high school student research at Washington University. Some strains used in this study were provided by the *Caenorhabditis* Genetics Center, which is supported by the National Institutes of Health's National Center for Research Resources.

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Received for publication July 9, 1998
Accepted August 13, 1998